



AFRL-AFOSR-VA-TR-2016-0002

---

**Mechanistic Basis for Biological Polymer Stability, Electron Transfer and Molecular Sensing in Extreme Environments**

**Matthew Posewitz  
COLORADO SCHOOL OF MINES**

---

**12/02/2015  
Final Report**

DISTRIBUTION A: Distribution approved for public release.

Air Force Research Laboratory  
AF Office Of Scientific Research (AFOSR)/ RTB2  
Arlington, Virginia 22203  
Air Force Materiel Command

<b>REPORT DOCUMENTATION PAGE</b>		Form Approved OMB No. 0704-0188	
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Executive Services, Directorate (0704-0188). Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p><b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.</b></p>			
<b>1. REPORT DATE (DD-MM-YYYY)</b> 03-12-2015		<b>2. REPORT TYPE</b> Final Performance	
		<b>3. DATES COVERED (From - To)</b> 01-09-2014 to 31-08-2015	
<b>4. TITLE AND SUBTITLE</b> Mechanistic Basis for Biological Polymer Stability, Electron Transfer and Molecular Sensing in Extreme Environments		<b>5a. CONTRACT NUMBER</b>	
		<b>5b. GRANT NUMBER</b> FA9550-14-1-0147	
		<b>5c. PROGRAM ELEMENT NUMBER</b> 61102F	
<b>6. AUTHOR(S)</b> Matthew Posewitz		<b>5d. PROJECT NUMBER</b>	
		<b>5e. TASK NUMBER</b>	
		<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> COLORADO SCHOOL OF MINES 1500 ILLINOIS ST GOLDEN, CO 80401-1887 US		<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> AF Office of Scientific Research 875 N. Randolph St. Room 3112 Arlington, VA 22203		<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b> AFRL/AFOSR RTB2	
		<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION/AVAILABILITY STATEMENT</b> A DISTRIBUTION UNLIMITED: PB Public Release			
<b>13. SUPPLEMENTARY NOTES</b>			
<b>14. ABSTRACT</b> <p>Research focused on the discovery of the determinants enabling thermal stability in electron transfer proteins. Crystal structures for two enzymes were determined. Mercuric ion reductase (MerA), a mercury detoxification enzyme, has been tuned by evolution to have high specificity for mercuric ions (Hg<sup>2+</sup>) and to catalyze their reduction to a more volatile, less toxic elemental form. Biochemical and structural characterization of MerA from the thermophilic crenarchaeon Metallosphaera sedula demonstrated that this is a thermostable enzyme that remains active after extended incubation at 97C. At 37C, the NADPH oxidation-linked Hg<sup>2+</sup> reduction specific activity was found to be 1.9 mol/minmg, increasing to 3.1 mol/minmg at 70C. M. sedula MerA crystals were obtained and the structure was solved to 1.6 Å, representing the first solved crystal structure of a thermophilic MerA. Comparison of both the crystal structure and amino acid sequence of MerA from M. sedula to mesophilic counterparts provides new insights into the structural determinants that underpin the thermal stability of the enzyme. Enolase catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during both glycolysis and gluconeogenesis, and is required by all three domains of life. Biochemical and structural characterization of enolase from Chloroflexus aurantiacus, a thermophilic anoxygenic phototroph affiliated with the green non-sulfur bacteria established a homodimer with a subunit molecular weight of 46 kDa. The temperature optimum for enolase catalysis was 80C, close to the measured thermal stability of the protein which was</p>			
<b>15. SUBJECT TERMS</b> thermally stable proteins, halophiles, acidophiles			

Standard Form 298 (Rev. 8/98)  
Prescribed by ANSI Std. Z39.18

DISTRIBUTION A: Distribution approved for public release

<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>  UU	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b> Matthew Posewitz
<b>a. REPORT</b>  Unclassified	<b>b. ABSTRACT</b>  Unclassified	<b>c. THIS PAGE</b>  Unclassified			<b>19b. TELEPHONE NUMBER</b> <i>(Include area code)</i> 303-384-2425

# Mechanistic Basis for Biological Polymer Stability, Electron Transfer and Molecular Sensing in Extreme Environments

FA9550-14-1-0147

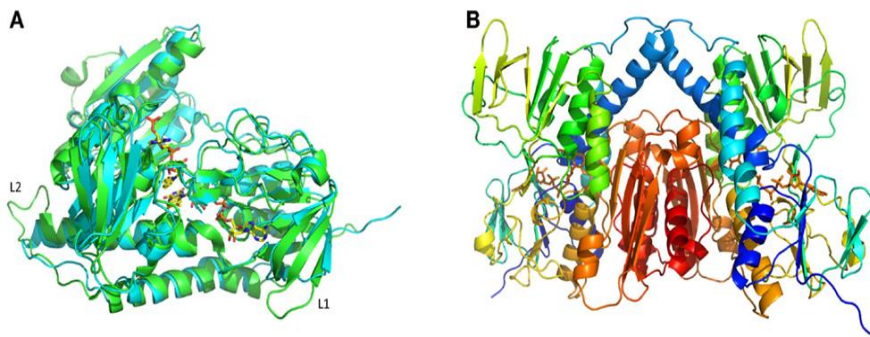
Matthew C. Posewitz (Colorado School of Mines)

John W. Peters (Montana State University)

Patrick C. Hallenbeck, Donald Veverka (U.S. Air Force Academy)

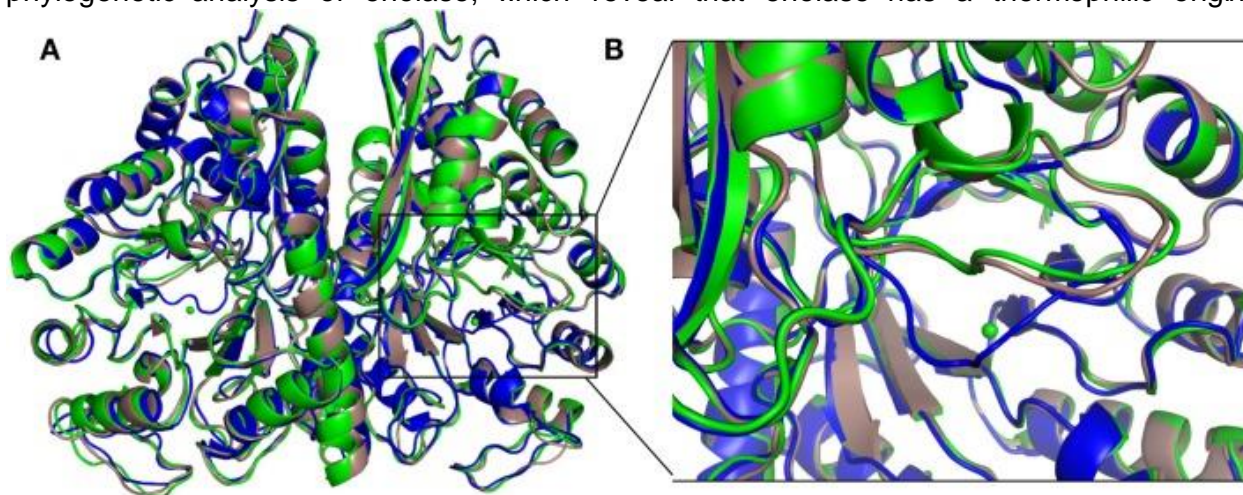
Research focused on the discovery of the determinants enabling thermal stability in electron transfer proteins. Four specific projects were pursued as summarized below (biochemical characterizations and structural analysis of two thermophilic enzymes, electron transfer reactions in cyanobacteria, and the first description of a thermophilic microbial fuel cell).

Crystal structures for two enzymes were determined. Mercuric ion reductase (MerA), a mercury detoxification enzyme, has been tuned by evolution to have high specificity for mercuric ions ( $\text{Hg}^{2+}$ ) and to catalyze their reduction to a more volatile, less toxic elemental form. Here, we performed biochemical and structural characterization of MerA from the thermophilic crenarchaeon *Metallosphaera sedula*. MerA from *M. sedula* is a thermostable enzyme, and remains active after extended incubation at 97°C. At 37°C, the NADPH oxidation-linked  $\text{Hg}^{2+}$  reduction specific activity was found to be 1.9  $\mu\text{mol}/\text{min}\cdot\text{mg}$ , increasing to 3.1  $\mu\text{mol}/\text{min}\cdot\text{mg}$  at 70°C. *M. sedula* MerA crystals were obtained and the structure was solved to 1.6 Å, representing the first solved crystal structure of a thermophilic MerA. Comparison of both the crystal structure and amino acid sequence of MerA from *M. sedula* to mesophilic counterparts provides new insights into the structural determinants that underpin the thermal stability of the enzyme.

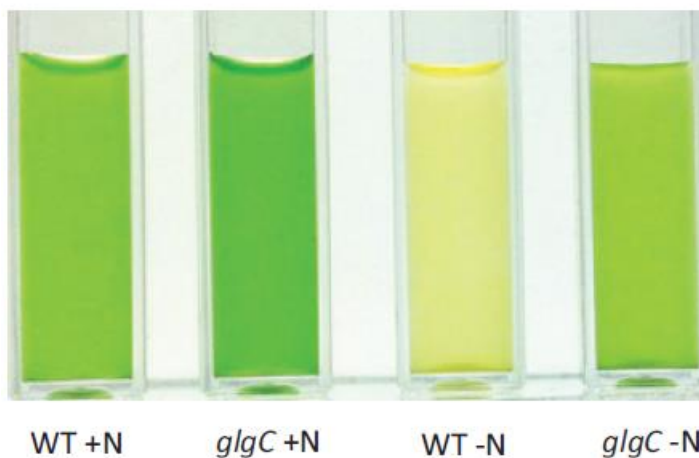


Enolase catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during both glycolysis and gluconeogenesis, and is required by all three domains of life. We report the purification and biochemical and structural characterization of enolase from *Chloroflexus aurantiacus*, a thermophilic anoxygenic phototroph affiliated with the green non-sulfur bacteria. The protein was purified as a homodimer with a subunit molecular weight of 46 kDa. The temperature optimum for enolase catalysis was 80°C, close to the measured thermal stability of the protein which was determined to be 75°C, while the pH optimum for enzyme activity was 6.5. The specific activities of purified enolase determined at 25 and 80°C were 147 and 300  $\text{U mg}^{-1}$  of protein, respectively.  $K_m$  values for the 2-phosphoglycerate/phosphoenolpyruvate reaction determined at 25 and 80°C were 0.16 and 0.03 mM, respectively. The  $K_m$  values for  $\text{Mg}^{2+}$  binding at these temperatures were 2.5 and 1.9 mM, respectively. When compared to enolase from mesophiles, the biochemical and structural properties of enolase from *C. aurantiacus* are consistent with this being thermally adapted. These data are consistent with the results of our

phylogenetic analysis of enolase, which reveal that enolase has a thermophilic origin.



Cyanobacterial glycogen-deficient mutants display impaired degradation of light-harvesting phycobilisomes under nitrogen-limiting growth conditions and secrete a suite of organic acids as a putative reductant-spilling mechanism. This genetic background, therefore, represents an important platform to better understand the complex relationships between light harvesting, photosynthetic electron transport, carbon fixation, and carbon/nitrogen metabolisms. In this study, we conducted a comprehensive analysis of the dynamics of photosynthesis as a function of reductant sink manipulation in a glycogen-deficient *glgC* mutant of *Synechococcus* sp. strain PCC 7002. The *glgC* mutant showed increased susceptibility to photoinhibition during the initial phase of nitrogen deprivation. However, after extended periods of nitrogen deprivation, *glgC* mutant cells maintained higher levels of photosynthetic activity than the wild type, supporting continuous organic acid secretion in the absence of biomass accumulation. In contrast to the wild type, the *glgC* mutant maintained efficient energy transfer from phycobilisomes to photosystem II (PSII) reaction centers, had an elevated PSII/PSI ratio as a result of reduced PSII degradation, and retained a nitrogen-replete-type ultrastructure, including an extensive thylakoid membrane network, after prolonged nitrogen deprivation. Together, these results suggest that multiple global signals for nitrogen deprivation are not activated in the *glgC* mutant, allowing the maintenance of active photosynthetic complexes under conditions where photosynthesis would normally be abolished.

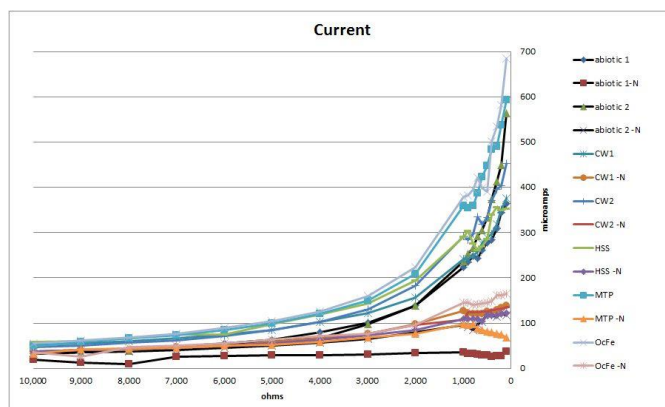
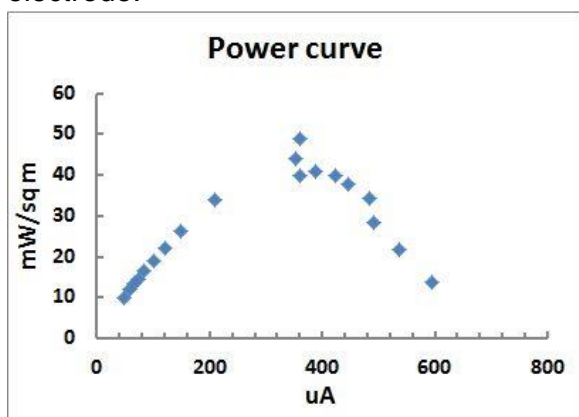
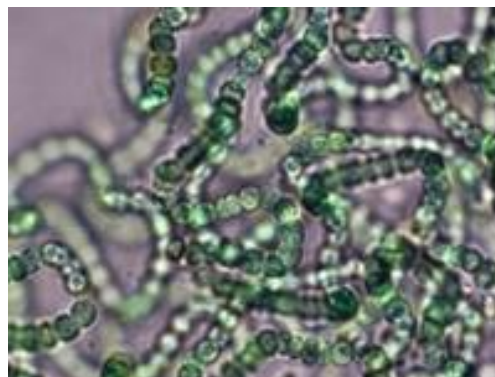


Many previous studies have shown that several different kinds of mesophilic organisms are capable of interacting with inorganic electrodes and giving up electrons derived from organic materials. This has led to research into the use of microbial fuel cells (MFC) for waste treatment and power generation, or to the use of microbial electrocatalysis for the electrosynthesis of chemicals and fuels, basically producing compounds of interest from electrically driven  $\text{CO}_2$  fixation. Many different types of extremophiles are known that are robust and resistant to heat or

pH extremes. These organisms have evolved unique metabolic adaptations to their environments and may have unique sensing capabilities.

The goals of the work carried out at USAFA were to discover and characterize electrically active extremophiles using both defined, characterized extremophiles and enriched environmental samples. These could potentially be used in the long term to develop robust miniaturized threat detection systems, for remote power generation and in general for developing systems with increased capabilities for organic-inorganic electrical connectivity.

A variety of enriched samples taken from thermophilic environments (hot springs) in Colorado and New Mexico, were tested. These consisted mostly of assemblages of green algae and cyanobacteria with associated bacteria. In some cases the number of organisms was restrained enough to allow the determination of genome sequences. Interestingly, one sample (OC1) was enriched for a novel acidobacterium previously only observed in Octopus Hot Springs in Yellowstone. In another case (MTP1), a unique (above the genus level) thermophilic cyanobacterium could be described. All environmental enrichments were tested in thermophilic MFCs and this last enrichment in particular showed relatively (compared to control and other cultures) high currents and gave a power curve that demonstrated real connectivity with the electrode.



These initial results are encouraging and represent one of the first demonstrations of activity in a thermophilic MFC. Future research will focus on determining the role in electron transfer of the organisms in this biofilm as well as the molecular mechanism(s) involved.

### Publications for the one year of funding:

Artz, J.H., White, S.N., Zadvorny, O.A., Fugate, C.J., Hicks, D., Gauss, G.H., Posewitz, M.C., Boyd, E.S., and Peters, J.W. (2015) Biochemical and Structural Properties of a Thermostable Mercuric Ion Reductase from *Metallosphaera sedula*. *Frontiers in Bioengineering and Biotechnology* Jul 13;3:97. doi: 10.3389/fbioe.2015.00097.

Zadvorny O.E., Boyd, E.S., Posewitz, M.C., Zorin, N.A., and Peters, J.W. (2015) Biochemical and structural characterization of enolase from *Chloroflexus aurantiacus*: Evidence for a thermophilic origin. *Frontiers in Bioengineering and Biotechnology* Jun 1;3:74. doi: 10.3389/fbioe.2015.00074.

Jackson, S.A., Eaton-Rye, J.J., Bryant, D.A., Posewitz, M.C., Davies, F.K. (2015) Dynamics of photosynthesis in the glycogen-deficient *glgC* mutant of *Synechococcus* sp. PCC 7002. *Applied and Environmental Microbiology*, **81**, 6210-22. doi: 10.1128/AEM.01751-15.

Krishnan, A., Kumaraswamy, G.K., Vinyard, D.J., Gu, H., Ananyev, G., Posewitz, M.C., and Dismukes, G.C. (2015) Metabolic and photosynthetic consequences of blocking starch biosynthesis in the green alga *Chlamydomonas reinhardtii* *sta6* mutant. *Plant Journal* **81**, 947-960.

D'Adamo, S., and Posewitz, M.C. (2015) Hydrogenase evolution and function in eukaryotic algae. In: Low-Oxygen Stress in Plants; Plant Cell Monographs, M. Roegner (Ed.), De Gruyter, Berlin, in press. pp. 147-174.

Hallenbeck, P.C., Grogger, M., Veverka, D. (2014) Recent Advances in Microbial Electrocatalysis. *Electrocatalysis* **5**, 319–329.

Hallenbeck, P.C., Grogger, M., Mraz, M., Veverka, D. (2015) Draft Genome of a thermophilic photoheterotrophic Chloracidobacterium thermophilum strain OC1 from Ojo Caliente, New Mexico, submitted to *Genome Announcements*.

Hallenbeck, P.C., Grogger, M., Mraz, M., Veverka, D. (2015) Draft Genome of a thermophilic cyanobacterium from the family Oscillatoriales (strain MTP1) from the Chalk River, Colorado, submitted to *Genome Announcements*.

1.

**1. Report Type**

Final Report

**Primary Contact E-mail****Contact email if there is a problem with the report.**

mposewit@mines.edu

**Primary Contact Phone Number****Contact phone number if there is a problem with the report**

303-384-2425

**Organization / Institution name**

Colorado School of Mines

**Grant/Contract Title****The full title of the funded effort.**

Mechanistic Basis for biological polymer stability in extreme environments

**Grant/Contract Number****AFOSR assigned control number. It must begin with "FA9550" or "F49620" or "FA2386".**

FA9550-14-1-0147

**Principal Investigator Name****The full name of the principal investigator on the grant or contract.**

Matthew Posewitz

**Program Manager****The AFOSR Program Manager currently assigned to the award**

Hugh DeLong

**Reporting Period Start Date**

09/01/2014

**Reporting Period End Date**

08/31/2015

**Abstract**

Research focused on the discovery of the determinants enabling thermal stability in electron transfer proteins. Crystal structures for two enzymes were determined. Mercuric ion reductase (MerA), a mercury detoxification enzyme, has been tuned by evolution to have high specificity for mercuric ions ( $\text{Hg}^{2+}$ ) and to catalyze their reduction to a more volatile, less toxic elemental form. Biochemical and structural characterization of MerA from the thermophilic crenarchaeon *Metallosphaera sedula* demonstrated that this is a thermostable enzyme that remains active after extended incubation at  $97^\circ\text{C}$ . At  $37^\circ\text{C}$ , the NADPH oxidation-linked  $\text{Hg}^{2+}$  reduction specific activity was found to be  $1.9 \mu\text{mol}/\text{min}\cdot\text{mg}$ , increasing to  $3.1 \mu\text{mol}/\text{min}\cdot\text{mg}$  at  $70^\circ\text{C}$ . *M. sedula* MerA crystals were obtained and the structure was solved to  $1.6 \text{ \AA}$ , representing the first solved crystal structure of a thermophilic MerA. Comparison of both the crystal structure and amino acid sequence of MerA from *M. sedula* to mesophilic counterparts provides new insights into the structural determinants that underpin the thermal stability of the enzyme. Enolase catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during both glycolysis and gluconeogenesis, and is required by all three domains of life. Biochemical and structural characterization of enolase from *Chloroflexus aurantiacus*, a thermophilic anoxygenic phototroph affiliated with the green non-sulfur bacteria established a homodimer with a subunit molecular weight of 46 kDa. The temperature optimum for enolase catalysis was  $80^\circ\text{C}$ , close to the measured thermal stability of the protein which was

determined to be 75 °C, while the pH optimum for enzyme activity was 6.5. The specific activities of purified enolase determined at 25 and 80 °C were 147 and 300 U mg<sup>-1</sup> of protein, respectively. Km values for the 2-phosphoglycerate/phosphoenolpyruvate reaction determined at 25 and 80 °C were 0.16 and 0.03 mM, respectively. The Km values for Mg<sup>2+</sup> binding at these temperatures were 2.5 and 1.9 mM, respectively. When compared to enolase from mesophiles, the biochemical and structural properties of enolase from *C. aurantiacus* are consistent with this being thermally adapted. These data are consistent with the results of our phylogenetic analysis of enolase, which reveal that enolase has a thermophilic origin. Lastly, novel microorganisms were demonstrated to be active in a thermophilic microbial fuel cell for the first time. In sum, eight peer reviewed manuscripts were published/submitted in the last year.

#### **Distribution Statement**

This is block 12 on the SF298 form.

Distribution A - Approved for Public Release

#### **Explanation for Distribution Statement**

If this is not approved for public release, please provide a short explanation. E.g., contains proprietary information.

#### **SF298 Form**

Please attach your SF298 form. A blank SF298 can be found [here](#). Please do not password protect or secure the PDF. The maximum file size for an SF298 is 50MB.

[AFD-070820-035-MP.pdf](#)

**Upload the Report Document. File must be a PDF. Please do not password protect or secure the PDF. The maximum file size for the Report Document is 50MB.**

[Report.pdf](#)

**Upload a Report Document, if any. The maximum file size for the Report Document is 50MB.**

#### **Archival Publications (published) during reporting period:**

Eight publications resulted from the reporting period:

Artz, J.H., White, S.N., Zadvorny, O.A., Fugate, C.J., Hicks, D., Gauss, G.H., Posewitz, M.C., Boyd, E.S., and Peters, J.W. (2015) Biochemical and Structural Properties of a Thermostable Mercuric Ion Reductase from *Metallosphaera sedula*. *Frontiers in Bioengineering and Biotechnology* Jul 13;3:97. doi: 10.3389/fbioe.2015.00097.

Zadvorny O.E., Boyd, E.S., Posewitz, M.C., Zorin, N.A., and Peters, J.W. (2015) Biochemical and structural characterization of enolase from *Chloroflexus aurantiacus*: Evidence for a thermophilic origin. *Frontiers in Bioengineering and Biotechnology* Jun 1;3:74. doi: 10.3389/fbioe.2015.00074.

Jackson, S.A., Eaton-Rye, J.J., Bryant, D.A., Posewitz, M.C., Davies, F.K. (2015) Dynamics of photosynthesis in the glycogen-deficient glgC mutant of *Synechococcus* sp. PCC 7002. *Applied and Environmental Microbiology*, 81, 6210-22. doi: 10.1128/AEM.01751-15.

Krishnan, A., Kumaraswamy, G.K., Vinyard, D.J., Gu, H., Ananyev, G., Posewitz, M.C., and Dismukes, G.C. (2015) Metabolic and photosynthetic consequences of blocking starch biosynthesis in the green alga *Chlamydomonas reinhardtii* sta6 mutant. *Plant Journal* 81, 947-960.

D'Adamo, S., and Posewitz, M.C. (2015) Hydrogenase evolution and function in eukaryotic algae. In: *Low-Oxygen Stress in Plants; Plant Cell Monographs*, M. Roegner (Ed.), De Gruyter, Berlin, in press. pp. 147-174.

Hallenbeck, P.C., Grogger, M., Veverka, D. (2014) Recent Advances in Microbial Electrocatalysis. *Electrocatalysis* 5, 319–329.

Hallenbeck, P.C., Grogger, M., Mraz, M., Veverka, D. (2015) Draft Genome of a thermophilic  
DISTRIBUTION A: Distribution approved for public release

photoheterotrophic Chloracidobacterium thermophilum strain OC1 from Ojo Caliente, New Mexico, submitted to Genome Announcements.

Hallenbeck, P.C., Grogger, M., Mraz, M., Veverka, D. (2015) Draft Genome of a thermophilic cyanobacterium from the family Oscillatoriales (strain MTP1) from the Chalk River, Colorado, submitted to Genome Announcements.

**Changes in research objectives (if any):**

None

**Change in AFOSR Program Manager, if any:**

Patrick Bradshaw to Hugh DeLong

**Extensions granted or milestones slipped, if any:**

None

**AFOSR LRIR Number**

**LRIR Title**

**Reporting Period**

**Laboratory Task Manager**

**Program Officer**

**Research Objectives**

**Technical Summary**

**Funding Summary by Cost Category (by FY, \$K)**

	Starting FY	FY+1	FY+2
Salary			
Equipment/Facilities			
Supplies			
Total			

**Report Document**

**Report Document - Text Analysis**

**Report Document - Text Analysis**

**Appendix Documents**

**2. Thank You**

**E-mail user**

Nov 17, 2015 08:07:09 Success: Email Sent to: mposewit@mines.edu